

material (see Figure 22). The microporous material should have a pore size sufficiently large to allow the nanoparticles to pass through the pores and sufficiently small to retain the network on the surface of the microporous material when the microporous material is washed (see Figure 22). Many suitable microporous materials are known in the art and include those described above. Such a microporous material retains the network composed of target nucleic acid and the two probes, and a positive result (presence of the target nucleic acid) is evidenced by a lack of fluorescence (due to quenching of fluorescence by the metallic or semiconductor nanoparticles) (see Figure 22). A negative result (no target nucleic acid present) is evidenced by fluorescence because the nanoparticles would pass through the pores of the microporous material when it is washed (so no quenching of the fluorescence would occur) (see Figure 22). There is low background fluorescence because unbound probes are washed away from the detection area. In addition, in the case of a positive result, changes in fluorescence can be observed as a function of temperature. For instance, as the temperature is raised, fluorescence will be observed once the dehybridization temperature has been reached. Therefore, by looking at fluorescence as a function of temperature, information can be obtained about the degree of complementarity between the oligonucleotide probes and the target nucleic acid. Fluorescence can be generated by simply illuminating the solution or microporous material with a UV lamp, and the fluorescent signal can be monitored by the naked eye. Alternatively, for a more quantitative result, a fluorimeter can be employed in front-face mode to measure the fluorescence of the solution with a short path length.

In yet other embodiments, a “satellite probe” is used (see Figure 24). The satellite probe comprises a central particle with one or several physical properties that can be exploited for detection in an assay for nucleic acids (*e.g.*, intense color, fluorescence quenching ability, magnetism). Suitable particles include the nanoparticles and other particles described above. The particle has oligonucleotides (all having the same sequence) attached to it (see Figure 24). Methods of attaching oligonucleotides to the particles are described above. These oligonucleotides comprise at least a first portion and a second

portion, both of which are complementary to portions of the sequence of a target nucleic acid (see Figure 24). The satellite probe also comprises probe oligonucleotides. Each probe oligonucleotide has at least a first portion and a second portion (see Figure 24). The sequence of the first portion of the probe oligonucleotides is complementary to the first portion of the sequence of the oligonucleotides immobilized on the central particle (see Figure 24). Consequently, when the central particle and the probe oligonucleotides are brought into contact, the oligonucleotides on the particle hybridize with the probe oligonucleotides to form the satellite probe (see Figure 24). Both the first and second portions of the probe oligonucleotides are complementary to portions of the sequence of the target nucleic acid (see Figure 24). Each probe oligonucleotide is labeled with a reporter molecule (see Figure 24), as further described below. The amount of hybridization overlap between the probe oligonucleotides and the target (length of the portion hybridized) is as large as, or greater than, the hybridization overlap between the probe oligonucleotides and the oligonucleotides attached to the particle (see Figure 24). Therefore, temperature cycling resulting in dehybridization and rehybridization would favor moving the probe oligonucleotides from the central particle to the target. Then, the particles are separated from the probe oligonucleotides hybridized to the target, and the reporter molecule is detected.

The satellite probe can be used in a variety of detection strategies. For example, if the central particle has a magnetic core and is covered with a material capable of quenching the fluorescence of fluorophores attached to the probe oligonucleotides that surround it, this system can be used in an *in situ* fluorometric detection scheme for nucleic acids. Functionalized polymer-coated magnetic particles ( $\text{Fe}_3\text{O}_4$ ) are available from several commercial sources including Dynal (Dynabeads<sup>TM</sup>) and Bangs Laboratories (Estapor<sup>TM</sup>), and silica-coated magnetic  $\text{Fe}_3\text{O}_4$  nanoparticles could be modified (Liu et al., *Chem. Mater.*, **10**, 3936-3940 (1998)) using well-developed silica surface chemistry (Chrissey et al., *Nucleic Acids Research*, **24**, 3031-3039 (1996)) and employed as magnetic probes as well. Further, the dye molecule, 4-((4-(dimethylamino)phenyl)-azo)benzoic acid (DABCYL) has been shown to be an efficient quencher of fluorescence for a wide variety of fluorophores attached

to oligonucleotides (Tyagi et al., *Nature Biotech.*, **16**, 49-53 (1998). The commercially-available succinimidyl ester of DABCYL (Molecular Probes) forms extremely stable amide bonds upon reaction with primary alkylamino groups. Thus, any magnetic particle or polymer-coated magnetic particle with primary alkyl amino groups could be modified with both oligonucleotides, as well as these quencher molecules. Alternatively, the DABCYL quencher could be attached directly to the surface-bound oligonucleotide, instead of the alkyl amino-modified surface. The satellite probe comprising the probe oligonucleotides is brought into contact with the target. The temperature is cycled so as to cause dehybridization and rehybridization, which causes the probe oligonucleotides to move from the central particle to the target. Detection is accomplished by applying a magnetic field and removing the particles from solution and measuring the fluorescence of the probe oligonucleotides remaining in solution hybridized to the target.

This approach can be extended to a colorimetric assay by using magnetic particles with a dye coating in conjunction with probe oligonucleotides labeled with a dye which has optical properties that are distinct from the dye on the magnetic nanoparticles or perturb those of the dye on the magnetic nanoparticles. When the particles and the probe oligonucleotides are in solution together, the solution will exhibit one color which derives from a combination of the two dyes. However, in the presence of a target nucleic acid and with temperature cycling, the probe oligonucleotides will move from the satellite probe to the target. Once this has happened, application of a magnetic field will remove the magnetic, dye-coated particles from solution leaving behind probe oligonucleotides labeled with a single dye hybridized to the target. The system can be followed with a colorimeter or the naked eye, depending upon target levels and color intensities.

This approach also can be further extended to an electrochemical assay by using an oligonucleotide-magnetic particle conjugate in conjunction with a probe oligonucleotide having attached a redox-active molecule. Any modifiable redox-active species can be used, such as the well-studied redox-active ferrocene derivative. A ferrocene derivatized phosphoramidite can be attached to oligonucleotides directly using standard phosphoramidite